Stress Proteins in Reproductive Toxicology

David J. Dix

Reproductive Toxicology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 USA

Although a wide range of physical and chemical agents disrupt gametogenesis and embryogenesis in animals and humans, much less is known about the mechanisms leading up to these disruptions and the factors that determine whether a particular animal or human will be sensitive to a potential toxicant. Recent research has identified a number of different stress protein families, also known as heat shock proteins (HSPs), that play a role in protecting cells and organs from the toxic effects of exposure to hyperthermia and chemical insult (*1*). These stress proteins could provide significant insight into cellular processes susceptible to toxicant action and the mechanisms that have evolved to protect them. Understanding these protective mechanisms will allow more accurate extrapolation of animal data to humans and thereby improve the assessment of human health risk for a potentially broad range of reproductive and developmental toxicants.

To explore the role of stress proteins in reproductive toxicology, the Reproductive Toxicology Division of the National Health and Environmental Effects Research Laboratory of the U.S. Environmental Protection Agency (EPA) held a mini-workshop on 1 November 1996 in Research Triangle Park, North Carolina. Seven intramural and extramural scientists (see shaded box), experts in the field of HSPs, presented findings from their respective laboratories and participated in a roundtable discussion of research issues significant to the workshop theme. The research and its implications for toxicology are summarized here. This information promises to provide insights into toxicological processes modulated by HSPs. Furthermore, this research will advance the EPA's long-term goal to develop a scientific basis for assessing and characterizing risks to human health.

Mammalian Heat Shock Proteins Relevant to Reproductive Toxicology

Stress proteins are typically categorized by molecular mass into families of homologous genes and proteins (Table 1). In gametes and embryos, exposure to a wide range of toxic agents results in the increased expression and/or subcellular translocation of 27-, 70-, 90-, and 105-kDa HSPs (*2–7*). This workshop included reports on efforts to characterize the expression and function of stress proteins in rodent gametes and embryos, to create transgenic models wherein the expression of HSPs has been altered, and to use these models to understand biological processes such as cell cycle control and apoptotic cell death, which involve stress proteins and are significant to reproductive toxicology.

HSP27. HSP27 is constitutively expressed in most mammalian cells; however, the level of HSP27 expression and phosphorylation status of the protein can be increased by exposure to heat and other toxicants (*6*). In rat testes, *Hsp27* expression varies with the cycle of the seminiferous epithelium, and HSP27 protein is associated with microfilaments in Sertoli cells (*7*). *Hsp27* expression is low in seminiferous tubules at stages that are differentially sensitive to agents that disrupt spermatogenesis (e.g., cadmium), and this sensitivity may be related to the degree of HSP27 stabilization of Sertoli cell microfilaments at different stages of the spermatogenic cycle. The level of HSP27 expression has also been positively correlated with embryonic stem cell vulnerability to heat and metal toxicity (*8*), and similar correlations between vulnerability to toxicants and HSP27 have also been made in rat embryos exposed to hyperthermia (*6*). Thus, HSP27 appears to be involved in the development of thermotolerance and sensitivity to heat and chemical exposures in rodent testes and embryos. Whether HSP27 stabilization of microfilaments in Sertoli cells is related to thermotolerance remains to be proven.

HSP70s. HSP70s are the most widely studied and well defined of the stress protein families. Multiple *Hsp70* genes and encoded proteins have been characterized in mammals (*9–11*) and the expression of stress-inducible HSP70s is linked with exposure to a range of toxic agents including heat, sodium salicylate, and arsenic (*5*). Furthermore, in the rat whole-embryo culture model, induction and nuclear translocation of stress-inducible HSP70s positively correlates with both the onset and duration of thermotolerance (*12–13*). Whether the stress-inducible HSP70-1 and 70-3 are necessary and sufficient to prevent arsenite-induced neural tube defects in mouse embryos has been tested in a series of loss-of-function/gain-of-function experiments (*14*). Antisense inhibition of HSP70-1 and 70-3 expression resulted in an eightfold-higher incidence of neural tube defects in embryos exposed to normally subteratogenic doses of arsenite. Gain of HSP function was accomplished using a constitutive-promoter transgene construct that overexpressed HSP70-1 and significantly reduced embryo sensitivity to arsenite-induced neural tube defects (*14*). Thus, both direct and correlative data support the assertion that stress-inducible HSP70s protect embryos from the effects of toxicant exposure.

Besides the stress-inducible HSP70s, a number of different HSP70s are expressed either constitutively or during specific developmental phases in gametes and embryos (*15*). One example of developmental regulation of a heat shock protein is *Hsp70-2* expression in mouse spermatocytes (*16*). To determine if HSP70-2 has a critical role in meiotic spermatogenesis, the *Hsp70-2* gene was disrupted in mice (*17*). Gene knockout of *Hsp70-2* resulted in pachytene spermatocytes that became apoptotic and arrested at the G_2/M-phase transition of meiosis I (*18*). Furthermore, the cyclin B1-CDC2 complex required for the G_2/M-phase transition failed to assemble and become an active kinase in the mutant spermatocytes (*19*). Thus, the phenotype of the *Hsp70-2* knockout mice indicates a previously unrecognized relationship between an HSP70, meiotic cell cycle, and germ cell apoptosis. The possibility of similar roles for other HSP70s in gametes and embryos is an area of active research because maintenance of the cell cycle and appropriate apoptosis are critical to normal development.

Other HSPs. Exposure of gametes and embryos to toxic agents results in the induced expression of a number of other HSPs in addition to HSP27 and HSP70s. *In vivo* and *in vitro* exposure of organogenesis-stage rat or mouse embryos to toxic doses of heat or arsenic induces expression not only of

Address correspondence to D.J. Dix, MD-72, RTD/NHEERL, U.S. EPA, Research Triangle Park, NC 27711 USA.

The author thanks Robert Kavlock (Director, Reproductive Toxicology Division) for supporting this workshop and Teresa Wall for excellent administrative assistance. This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication.

Received 21 November 1996; accepted 8 January 1997.

Table 1. Mammalian heat shock proteins (HSPs)

HSP families[a]	Description and expression
HSP10	GroES-homologue, chaperonin
HSP27	Developmental expression in testes, inducible in embryos
HSP40	DNAJ-homologue, inducible in reproductive tissues
HSP60	GroEL-homologue, TCP1 chaperonin
HSP70	DNAK-homologue, chaperonin; binds steroid receptors; constitutive, developmental, and inducible expression in reproductive tissues
HSP90	Binds steroid receptors, tyrosine kinases; constitutive, developmental, and inducible expression in reproductive tissues
HSP105	Inducible expression in reproductive tissues

[a]Most HSP families contain multiple genes/proteins (e.g., there are at least seven different mammalian HSP70s).

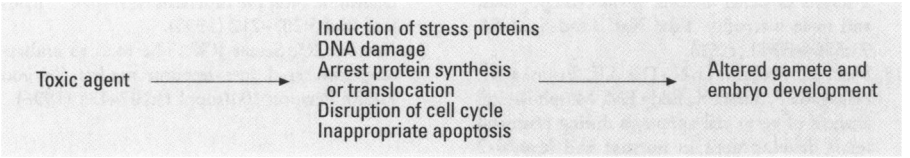

Figure 1. Exposure to toxic agents induces a range of biological effects in gametes and embryos, including the induction of stress or heat shock proteins (HSPs). Understanding the significance of HSP induction relative to the other biological effects of exposure may provide insights into the mechanisms leading from exposure to adverse developmental outcomes.

HSP70s but also HSP90 and HSP105 (*3–4, 20*). However, the connection between expression of different classes of HSPs in the same reproductive tissues is currently unknown, beyond the interaction of HSP90s with HSP70s during complexation with steroid receptors. The expression of HSP70 partner proteins such as the DNAJ-homologue HSP40 have been linked to the development of thermotolerance in somatic cells (*21*), and an HSP47 has been identified, which is constitutively expressed and heat-inducible in the developing neural plate of rat embryos (*22*). Whether this HSP47 is a DNAJ-homologue or whether it interacts with HSP70s (*23*) remains to be determined.

Future Research

Future research will attempt to address the following questions:

- In what biological processes do HSPs participate and how does this participation translate into protective mechanisms significant to reproductive toxicology? Exposure to toxic agents induces a range of biological effects that include the induction of stress proteins (Fig. 1). Transgenic animal gain-of-function and loss-of-function models should prove useful in testing the relationships between the expression of specific HSPs and other biological effects of exposure, and determining how these biological effects translate into adverse reproductive or developmental outcomes.
- What is the relationship between different families of HSPs in modulating reproductive and developmental toxicity? Determining whether different HSPs protect the same biological processes and whether HSPs provide additive or synergistic effects will be significant to developing complete models of *in vivo* systems. Various combinations of HSP transgenic models will be useful in understanding how different types of HSPs interact with each other in gametes and embryos.
- How will understanding HSP function be applied to estimating human health risks? Will it be possible to build quantitative models of toxicant action based upon HSPs as a common pathway? Conducting and assimilating mechanistic research is part of the EPA's effort to improve animal-to-human extrapolation and build biologically based dose–response models for use in risk assessment (*24*). Expanding and deepening our understanding of HSP functions will help in developing generalized mathematical models of the progression of toxicity.

References

1. Welch WJ. Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol Rev 72:1063–1081 (1992).
2. Allen RL, O'Brien DA, Jones CC, Rockett DL, Eddy EM. Expression of heat shock proteins by isolated mouse spermatogenic cells. Mol Cell Biol 8:3260–3266 (1988).
3. Kimmel CA, Kimmel GL, Lu C, Heredia DJ. Stress protein synthesis as a potential biomarker for heat-induced developmental toxicity. Teratology 43:465 (1991).
4. Honda K, Hatayama T, Takahashi K, Yukioka M. Heat shock proteins in human and mouse embryonic cells after exposure to heat shock or teratogenic agents. Teratog Carcinog Mutagen 11:235–244 (1992).
5. Mirkes PE, Doggett B, Cornel L. Induction of a heat shock response (HSP72) in rat embryos exposed to selected chemical teratogens. Teratology 49:135–142 (1994).
6. Mirkes PE, Little SA, Cornel L, Welsh MJ, Laney TN, Wright, FH. Induction of heat shock protein 27 in rat embryos exposed to hyperthermia. Mol Reprod Dev 25:276–284 (1996).
7. Welsh MJ, Wu W, Parvinen M, Gilmont RR. Variation in expression of hsp27 messenger ribonucleic acid during the cycle of the seminiferous epithelium and co-localization of hsp27 and microfilaments in Sertoli cells of the rat. Biol Reprod 55:141–151 (1996).
8. Wu W, Welsh MJ. Expression of the 25-kDa heat shock protein (HSP27) correlates with resistance to the toxicity of cadmium chloride, mercuric chloride, cis-platinum(II)-diammine dichloride, or sodium arsenite in mouse embry-

Sponsor
U.S. EPA, National Health and Environmental Effects Research Laboratory (NHEERL), Reproductive Toxicology Division (RTD), Research Triangle Park, North Carolina

Organizer
David Dix
Gamete and Early Embryo Biology Branch
RTD/NHEERL/U.S. EPA
Research Triangle Park, North Carolina

Participants
Clayton Hunt
Section of Cancer Biology
Washington University
St Louis, Missouri

E. Sidney Hunter III
Developmental Biology Branch
RTD/NHEERL/U.S. EPA
Research Triangle Park, North Carolina

Carole Kimmel
Nat'l Ctr for Environmental Assessment
U.S. EPA
Washington, D.C.

Gary Kimmel
Nat'l Ctr for Environmental Assessment
U.S. EPA
Washington, D.C.

Philip Mirkes
Department of Pediatrics
University of Washington
Seattle, Washington

Michael Welsh
Department of Anatomy and Cell Biology
University of Michigan
Ann Arbor, Michigan

onic stem cells transfected with sense or antisense HSP27 cDNA. Toxicol Appl Pharmacol 141:330–339 (1996).
9. Hunt C, Morimoto RI. Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70. Proc Natl Acad Sci USA 82:6455–6459 (1985).
10. Hunt C, Calderwood S. Characterization and sequence of a mouse hsp70 gene and its expression in mouse cell lines. Gene 87:199–204 (1990).
11. Hunt CR, Gasser DL, Chaplin DD, Pierce JC, Kozak CA. Chromosomal localization of five murine HSP70 gene family members: Hsp70-1, Hsp70-2, Hsp70-3, Hsc70t, and Grp78. Genomics 16:193–198 (1993).
12. Mirkes PE, Doggett B. Accumulation of heat shock protein 72 (hsp 72) in postimplantation rat embryos after exosure to various periods of hyperthermia (40°–43°C) in vitro: evidence that heat shock protein 72 is a biomarker of heat-induced embryotoxicity. Teratology 46: 301–309 (1992).
13. Thayer JM, Mirkes PE. Induction of Hsp 72 and transient nuclear localization of Hsc 73 and Hsp 72 correlate with the acquisition and loss of thermotolerance in postimplantation rat embryos. Dev Dyn (in press).
14. Hunter ES, Dix DJ. Antisense oligonucleotides against *Hsp70-1* and *70-3* increase mouse embryonic sensitivity to arsenite-induced dysmorphogenesis *in vitro*. Teratology 53:86 (1996).
15. Dix DJ. Hsp70 expression and function during gametogenesis. Cell Stress Chaperones (in press).
16. Dix DJ, Rosario-Herrle MO, Gotoh H, Mori C, Goulding EH, Voelker CR, Eddy EM. Developmentally regulated expression of Hsp70-2 and a Hsp70-2/lacZ transgene during spermatogenesis. Dev Biol 174:310–321 (1996).
17. Dix DJ, Allen JW, Collins BW, Mori C, Nakamura N, Poorman-Allen P, Goulding EH, Eddy EM. Targeted gene disruption of Hsp70-2 results in failed meiosis, germ cell apoptosis, and male infertility. Proc Natl Acad Sci USA 93:3264–3268 (1996).
18. Mori C, Nakamura N, Dix DJ, Fujioka M, Nakagawa S, Shiota K, Eddy EM. Morphological analysis of germ cell apoptosis during postnatal testis development in normal and Hsp70-2 knockout mice. Dev Dyn 208:125–136 (1997).
19. Zhu D, Dix DJ, Eddy EM. HSP70-2 is required for CDC2 kinase activity in meiosis I of mouse spermatocytes. J Mol Cell Biol 7: 357a (1996).
20. Fisher BR, Kimmel GL, Kimmel CA, Heredia DJ. The association of heat-induced alterations in protein synthesis with somite defects in rat embryos. Teratology 43:465 (1991).
21. Kaneko R, Hattori H, Hayashi Y, Tohnai I, Ueda M, Ohtsuka K. Heat-shock protein 40, a novel predictor of thermotolerance in murine cells. Radiat Res 142:91–97 (1995).
22. Li Z, Wu Y, Nagata K, Edwards MJ, Walsh DA. The role of HSP47 during early neural plate development. Teratology 51:163 (1995).
23. Rassow J, Voos W, Pfanner N. Partner proteins determine multiple functions of Hsp70. Trends Cell Biol 5:207–212 (1995).
24. Kavlock RJ, Setzer RW. The road to embryologically based dose–response models. Environ Health Perspect 104(suppl 1):107–121 (1996).